Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study—a randomized controlled trial1,2,3,4

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2Supported by a grant from Mars Inc. Mars also supplied the standardized powdered cocoa drinks used in this investigation. This is a free access article, distributed under terms (http://www.nutrition.org/publications/guidelines-and-policies/license/) that permit unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

3Supplemental Table 1 is available from the “Supplemental data” link in the online posting of the article and from the same link in the online table contents at http://ajcn.nutrition.org.

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Abstract

Background: Recent evidence has indicated that flavanol consumption may have many health benefits in humans, including improved cognitive activities.

Objective: The aim was to evaluate the effect of flavanol consumption on cognitive performance in cognitively intact elderly subjects.

Design: This was a double-blind, controlled, parallel-arm study conducted in 90 elderly individuals without clinical evidence of cognitive dysfunction who were randomly assigned to consume daily for 8 wk a drink containing 993 mg [high flavanol (HF)], 520 mg [intermediate flavanol (IF)], or 48 mg [low flavanol (LF)] of flavanols (CFs). Cognitive function was assessed at baseline and after 8 wk by using the Mini-Mental State Examination (MMSE), the Trail Making Test (TMT) A and B, and the Verbal Fluency Test (VFT).

Results: The changes in MMSE score in response to the 3 different treatments were not different. In contrast,
there was a positive impact of the intervention on specific aspects of cognitive function. Mean changes (±SEs) in the time required to complete the TMT A and B after consumption of the HF (−8.6 ± 0.4 and −16.5 ± 0.8 s, respectively) and IF (−6.7 ± 0.5 and −14.2 ± 0.5 s, respectively) drinks significantly (P < 0.0001) differed from that after consumption of the LF drinks (−0.8 ± 1.6 and −1.1 ± 0.7 s, respectively). Similarly, VFT scores significantly improved among all treatment groups, but the magnitude of improvement in the VFT score was significantly (P < 0.0001) greater in the HF group (7.7 ± 1.1 words/60 s) than in the IF (3.6 ± 1.2 words/60 s) and LF (1.3 ± 0.5 words/60 s) groups. Significantly different improvements in insulin resistance (P < 0.0001), blood pressure (P < 0.0001), and lipid peroxidation (P = 0.001) were also observed for the HF and IF groups in comparison with the LF group. Changes in insulin resistance explained ~17% of changes in composite z score (partial r² = 0.1703, P < 0.0001).

Conclusions: This dietary intervention study provides evidence that regular CF consumption can reduce some measures of age-related cognitive dysfunction, possibly through an improvement in insulin sensitivity. These data suggest that the habitual intake of flavanols can support healthy cognitive function with age. This trial was registered at www.controlled-trials.com as ISRCTN68970511.

Keywords: blood pressure, cocoa flavanols, cognitive function, insulin resistance, lipid peroxidation

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INTRODUCTION

The question of whether some degree of cognitive deterioration is an inevitable part of aging or should be considered as a pathological prestage of dementia is debated. Aging is usually accompanied by deficits in several domains of cognition, including speed of processing, working memory capacity, inhibitory processes, and long-term memory, whereas other aspects of cognitive function, such as implicit memory and knowledge storage are less influenced by aging (1). With increasing numbers of elderly adults, evidence-based approaches that can help to slow or prevent cognitive decline in the early stages of cognitive dysfunction are needed (2).

A number of factors have been proposed as potential contributors to brain aging, including hypertension and other cardiovascular disease risk factors (3, 4), as well as vascular changes, metabolic dysfunction, increased oxidative stress and enhanced inflammatory signaling within the aging brain, and nutritional factors (5, 6). Evidence suggestive of neuroprotective effects of flavonoids, polyphenolic compounds that are present in plant-based foods, has increased in recent years. Epidemiologic studies suggest a positive association between flavonoid intake and lower prevalence of cognitive impairment (7) and better cognitive performance (8, 9). Among the flavonoids, flavanols, a subclass abundant in tea, grapes, and cocoa products, have been proposed as having the potential to counteract many aspects of cognitive decline (10, 11). Data from animal and in vitro models show that flavonoids, including flavanols, can interact with the neuronal intracellular signaling pathways mediating neurodegeneration and neuroinflammation and with the cellular and molecular architecture of the brain responsible for memory, learning, and cognitive functions (10, 12). Evidence from human studies showed that the consumption of the specific mixture of flavanols found in cocoa can reduce blood pressure and improve insulin sensitivity (13, 14) and induce vasodilation of the peripheral (13, 14) and cerebral (15) vascular system increasing brain blood flow and perfusion (15, 16) mainly through an improvement in nitric oxide bioavailability in endothelial cells (17). These data suggest that the regular consumption of flavanols may have positive implications for age-related cognitive dysfunction. In support of this concept, in the Cocoa, Cognition, and A...
Cocoa flavanol consumption improves cognitive function, blood pressure... http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4340060/

(CoCoA) Study, the consumption of a drink enriched with cocoa flavanols (CFs) was shown to improve cognitive function in elderly adults with mild cognitive impairment (MCI) (18). Significant improvements in blood pressure and insulin resistance were also observed (18).

This second part of the CoCoA study was designed to test the hypothesis that the regular consumption of a CF-containing beverage would also be effective in improving cognitive performance in elderly subjects with evidence of cognitive dysfunction. The impact of this dietary modification on blood pressure, glucose metabolism, and oxidative stress was also studied given consistent evidence of positive effects of flavanols on these outcomes (13, 14, 19–21) and the potentially influential role of these outcomes on cognitive function (3).

**METHODS**

**Participants**

Participants were recruited among those referred to senior centers in the L’Aquila district, in central Italy. Criteria included participants who reported themselves as unconcerned about their own memory functions and with no clinically significant coexisting medical conditions, including known cardiovascular disease, cerebrovascular events, overt dementia [Mini-Mental State Examination (MMSE) score <27] or other neurological disorders, thyroid disorders, or inflammatory diseases. To avoid confounding due to the influence of concomitant depression on the performance on cognitive tests (22), subjects with a score on the Geriatric Depression Scale ≥11 were also excluded (23). Current smokers, habitual users of antioxidant supplements (including vitamins and E), habitual consumers of chocolate or other cocoa products (daily consumption of any amount), or individuals prescribed medications known to have antioxidant properties (including statins and glitazones) or interfere with cognitive functions (including benzodiazepines and antidepressants) were all excluded from participation. As long as participants were in stable condition, any other prescribed treatments were kept constant throughout the study period. Individuals with a BMI (in kg/m^2) >30 and subjects reporting weight change ±10 body weight within the past 6 mo before entering the study were also excluded. A total of 174 subjects were screened by evaluation of medical history, multidimensional assessment (including neuropsychological testing and physical examination), laboratory variables, and assessment of the individuals’ habitual diet (Figure 1). Sixty-six individuals did not meet eligibility criteria: 24 had vascular disease, 6 regularly used antioxidant-containing vitamin supplements, 5 were current smokers, 4 habitually consumed cocoa-based products, 15 had BMIs >30, 7 used statins, and 5 had MMSE scores <27. Eighteen individuals refused to participate. The remaining 90 individuals were enrolled in the study. The study was approved by the Ethics Committee of the Public Health Agency of L’Aquila, Italy, and written informed consent to participate was provided for each participant. This trial was registered at [www.controlled-trials.com](http://www.controlled-trials.com) as ISRCTN68970511.

**FIGURE 1**

Flow of participants through the phases of the study. MMSE, Mini-Mental State Examination.

**Study design and outcomes**

To investigate the impact of regular CF consumption on cognitive function in cognitively intact elderly individuals, an 8-wk double-blind, randomized, parallel-arm study was conducted between December 2006 and July 2008. After initial enrollment, study participants met with a dietitian to evaluate current dietary habits,
correct any nutritional insufficiencies, and discuss the incorporation of the low-calorie test products into their habitual diet. Participants were then instructed to maintain their usual lifestyle and intake of fruit and vegetables and to avoid or, to the maximum extent possible, limit the intake of specific flavanol-containing foods and beverages including tea, red wine, fruit and vegetable juices, and chocolate. A detailed list of foods was given to each participant to allow appropriate dietary choices without imposing restrictions on the total consumption of fruit, vegetables, and beverages (i.e., the total consumption of fruit and vegetables was not restricted; the recommendation was simply made to modify the types of these foods consumed). Dietary total flavanol intake was estimated by a validated semiquantitative dietary questionnaire (24) at the time of enrollment and after a 1-wk run-in period; total flavanol intake was recorded as the intake of free and gallated flavanol monomers as their structurally related oligomeric compounds known as proanthocyanidins. Results of this assessment are shown in Table 1; total flavanol intakes were in the range of what was previously reported for an Italian population (25, 26).

TABLE 1
Mean dietary total flavanol intakes at enrollment and after the 1-wk run-in period in the 3 treatment groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total Flavanol Intake (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrollment</td>
<td>48 mg</td>
</tr>
<tr>
<td>1-wk Run-in</td>
<td>520 mg</td>
</tr>
<tr>
<td>8 wk</td>
<td>993 mg</td>
</tr>
</tbody>
</table>

All participants were encouraged to continue with their usual physical activity throughout the study period. A 1-wk run-in period, participants were randomly assigned to consume once daily a dairy-based cocoa drink containing CFs either at high flavanol (HF; 993 mg flavanols/serving), intermediate flavanol (IF; 520 mg flavanols/serving), or low flavanol (LF; 48 mg flavanols/serving) amounts for 8 wk (Table 2). Throughout the intervention, drinks were provided in individual sachets containing a dry beverage mix; the mix was reconstituted with water according to provided instructions just before consumption. Frequent participant monitoring by weekly phone contact or prescheduled meetings, according to participant preference, was used to maximize compliance with the intervention and to minimize changes in overall diet and lifestyle. All participants were instructed to return excess and empty sachets to check compliance. Compliance was calculated by using amount of servings ingested divided by the amount the participants should have ingested and multiplied by 100.

TABLE 2
Chemical composition of the cocoa test drinks

<table>
<thead>
<tr>
<th>Ingredient (mg/serving)</th>
<th>HF (993 mg flavanols)</th>
<th>IF (520 mg flavanols)</th>
<th>LF (48 mg flavanols)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa flavanol content</td>
<td>1,000 mg</td>
<td>750 mg</td>
<td>3 mg</td>
</tr>
</tbody>
</table>

Randomization ensured that the groups at baseline were approximately equal, averaging out between-subject differences on potential confounding due to baseline nutritional habits. Neither the treating physicians nor the patients were aware of treatment allocation.

Main outcome measures examined were changes in cognitive function after 8 wk of regular CF consumption. Secondary outcome measures examined included changes in blood pressure, metabolic variables, and plasma markers of lipid peroxidation.

**Cocoa drinks**

The dairy-based cocoa drinks were supplied as a dry beverage mix in individual anonymized sachets. The test products used in this study were specially designed, calorically balanced, carefully nutrient- and alkaloid-
matched, and indistinguishable in appearance and had a flavanol content that was not obvious on the basis of flavor. The 3 beverage mixes contained similar macronutrient, mineral, theobromine, and caffeine content, varying significantly only in the content of CFs (Table 2). To achieve the higher content of flavanols, the HF::IF drink mixes were made with a flavanol-rich cocoa powder (Cocoapro processed cocoa powder; Mars Inc), whereas the LF drink was made with a highly processed, alkaliized cocoa powder. The flavanol composition of the cocoa drinks is also shown in Table 2. In this study, the term “cocoa flavanols” is used to define the sum of all monomeric flavanols and their oligomeric derivatives (procyanidins) up to and including decamers (10 monomeric subunits). To prepare the drinks, the contents of the packets were mixed by a hand-held mixer with 250 mL warm water and immediately consumed. The assigned drink was consumed once a day, in the morning during the entire length of the study, with the final drink consumed ~24 h before the final evaluation. Participants were instructed to promptly inform researchers of any adverse events, including intolerance.

Cognitive function assessment

Study participants arrived on the morning of evaluation after a small breakfast had been consumed at home before taking the daily cocoa drink. The participants were tested during a morning visit in a quiet room by neuropsychologically trained research assistants who were unaware of the aim of the study. The same research assistant administered the neuropsychological tests to the same subject during the study to minimize examinee bias. Cognitive testing was performed at baseline and after 8 wk (±2 d) by using a combination of 4 well-validated standardized tests: the MMSE (27), theTrail Making Test (TMT) A and B (28), and the verbal fluency test (VFT) (29). As per the predefined procedure, an integrated measure of overall cognitive function—composite cognitive z score—was also constructed for each participant by converting the log-transformed raw scores from the individual tests to standardized scores (z score) that were based on the means and pooled SDs of the whole cohort at baseline.

Blood pressure measurement

Before neuropsychological testing, clinical systolic and diastolic blood pressures were recorded in the morning with the use of a validated oscillometric device with appropriately sized cuffs (Omron 705 CP; Omron Matsusaka) on the nondominant upper arm. These evaluations were performed by staff blinded to the study protocol. At each visit, participants rested 15 min in a seated position, the first blood pressure measurement was taken but discarded, and the subsequent 3 consecutive blood pressure readings, taken at 3-min intervals, were recorded. The average of these latter measures was considered for statistical analysis.

Laboratory analysis

Within 24 h of neuropsychological testing, blood samples were drawn from each participant after an overnight fasting period for determinations of lipid profile and fasting plasma glucose and insulin. The HOMA-IR index: [fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5] was calculated from fasting glucose and insulin concentrations as a marker of insulin resistance. Plasma concentrations of total 8-iso-prostaglandin F$_2\alpha$ as an index of oxidative stress–related lipid peroxidation (30), were assessed by enzyme immunoassay (Assay Design Inc) according to the manufacturer’s instructions.

Statistical analysis

The statistical analysis was conducted according to the intention-to-treat principle, and a per-protocol analysis was performed as a sensitivity analysis. Accurate prestudy sample-size calculation was hindered by the lack of...
available data on the effect of CF administration on cognitive function in healthy elderly subjects. Thus, the study was based on an estimated sample size of 90 subjects, with a ratio 1:1:1 for the 3 treatment groups, which was calculated to be adequate to achieve 90% power to detect a moderate effect size (Cohen’s $f$: 0.25) with an $\alpha$ of 0.05 on the global cognitive $z$ score between HF and LF groups at week 8. SAS program version 9 was used to perform a 2-factor mixed-design ANOVA using the repeated statement with the general linear model procedure. Post hoc analysis between treatment groups was performed by Tukey’s studentized range honestly significant difference test. A 2-tailed $P < 0.05$ was considered significant. $\eta^2$ was computed as measure of the magnitude of treatment effects. A chi-square test was used to compare categorical variables; ANOVA was used for continuous variables. Spearman nonparametric correlation was used to evaluate correlations between variables. Change in composite cognitive $z$ score was entered as the dependent variable in a hierarchical multiple regression analysis in which changes in HOMA-IR, isoprostanes, and blood pressure were entered sequentially according to the pathophysiologic hypothesis of a driving effect of improvements in insulin resistance on cognitive outcomes. Analysis was performed on variables logarithmically transformed to enhance symmetry of measures. If not otherwise specified, data are presented as means ± SEs.

RESULTS

Study cohort

General characteristics of the study cohort are shown in Table 3. According to the selection criteria, none of the participants had a BMI >30, were current smokers, or were receiving statin treatment. Hypertension was the prevalent cardiovascular disease risk factor. The 3 study groups were comparable with regard to sex, age, anthropometric characteristics, cardiovascular disease risk factor prevalence, and pharmacologic treatments (Table 3, Table 4). Dietary total flavanol intake was also similar in the 3 study groups at enrollment and slightly but significantly decreased in the whole cohort during the run-in period to a similar degree in all of the group consequence of the dietary recommendations to limit as much as possible the intake of specific flavanol-containing foods, without significant differences between the 3 study groups at the end of follow-up (Table 1).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Baseline general characteristics of the study participants according to the assigned treatment</th>
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</table>

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Changes in body weight, BMI, blood pressure, and metabolic variables during the study period in the 3 treatment groups</th>
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</thead>
</table>

Adherence to the study protocol

One participant assigned to HF treatment discontinued beverage consumption after 3 wk because of personal reasons, one participant assigned to IF treatment discontinued beverage consumption after 3 wk because of reported gastric discomfort, and 2 participants assigned to LF treatment discontinued beverage consumption after 4 wk because of personal reasons. Aside from the single reported event of gastric discomfort, all intervention were well tolerated, with no other mild or more severe adverse events reported throughout the trial. All of the participants were followed up during the entire study period, and the data from the last scheduled measure according to the study protocol were included in the database for statistical analysis according to an intention...
to-treat procedure. The overall compliance, assessed by monitoring the number of returned drink sachets, was high, with compliance rates of 99.3% at week 4 and 98.7% at week 8, without any differences between the HF, IF, and LF interventions. Adherence to the dietary requirements was evaluated at randomization and at the end of follow-up by a checklist questionnaire of specified food items and by monitoring body weight. Compliance with the dietary restrictions was good because none of the subjects reported regular consumption of the restricted flavanol/procyanidin-containing foods and beverages. Furthermore, indications on overall diet and lifestyle were adequately followed by participants throughout the study because no significant changes in body weight or blood pressure were observed at the end of follow-up (Table 4).

Cognitive function measures

Baseline performance on the cognitive function tests was similar for the 3 treatment groups, indicating an adequate randomization procedure (Table 5). The response of MMSE scores to the 3 different treatments during the study period did not differ (P = 0.52) (Table 5). In contrast, there was an impact of the intervention on specific aspects of cognitive function. The changes throughout the study period in the time required to complete TMT A for HF and IF groups significantly differed from those for the LF group (P < 0.0001), with significant time reductions observed in subjects assigned to HF and IF interventions but not in those assigned to the LF intervention (Table 5). Similarly, the changes throughout the study period in the time required to complete TMT B for the HF and IF groups were significantly different in comparison to those for the LF group (P < 0.0001), with significant time reductions again observed among HF and IF subjects but not in those assigned to the LF intervention (Table 5).

<table>
<thead>
<tr>
<th>TABLE 5</th>
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<tr>
<td>Changes in neuropsychological test scores during the study period in the 3 treatment groups</td>
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</table>

VFT scores significantly improved in the study among all treatment groups, but to various degrees (P < 0.0001). Consistent with the TMT scores, significant improvements in verbal fluency were observed in the HF and IF groups. A significant improvement, although to a lesser extent than that seen in the HF and IF groups, was also observed after 8 wk among those assigned to the LF group; this was the only cognitive test for which a significant cognitive improvement was observed in this treatment group. The magnitude of improvement in VFT score was significantly greater in HF subjects in comparison to those assigned to the IF and LF groups (Table 5).

As an integrated measure of overall cognitive function, a composite overall z score was calculated. Consistent with the results detailed earlier, changes in the composite cognitive z score differed during the study period between the 3 treatment groups (P < 0.0001), with the HF and IF groups showing significant improvements after 8 wk of regular consumption of the defined intervention; no significant variations in the composite z score were observed in the LF group (Table 5). In a comparison across groups, despite no differences at baseline, the composite cognitive z score at the end of follow-up was significantly (P < 0.0001) better in subjects assigned the HF and IF interventions than at week 0 and was significantly (P < 0.05) better in comparison to those assigned to the LF intervention (Table 5).

η² Values as measures of the magnitude of treatment effects are presented as online supplemental material (Supplemental Table 1). All of the above results were confirmed by per protocol analysis (HF, n = 29; IF, n = 29; LF, n = 28) with the exception of the loss of statistical significance in the difference in the time required to complete TMT A for HF and IF groups significantly differed from those for the LF group (P < 0.0001), with significant time reductions observed in subjects assigned to HF and IF interventions but not in those assigned to the LF intervention (Table 5).
complete the TMT B at 8 wk between IF and LF groups (data not shown).

**Blood pressure and metabolic variables**

Baseline blood pressure and metabolic variables were similar for the 3 treatment groups (Table 4), with no significant differences in any of these variables between any of the groups at baseline.

**Blood pressure** The response of systolic blood pressure to the 3 treatments during the study period differed ($F_0.0001$), with significant reductions among HF and IF subjects (Table 4). Systolic blood pressure at the end of follow-up was significantly ($P < 0.05$) lower in subjects assigned to the HF and IF treatments compared with those assigned to the LF treatment (Table 4). The response of diastolic blood pressure to the 3 different treatments during the study period also differed ($P < 0.0001$), with significant reductions among HF and IF subjects (Table 4). A significant reduction in diastolic blood pressure was also observed in subjects assigned to the LF group, but this decrease was lower in magnitude than the changes observed in the HF and IF groups (Table 4). Diastolic blood pressure did not differ significantly between the 3 treatment groups at week 8 (Table 4).

**Metabolic variables** The response of plasma glucose and insulin to the 3 treatments during the study period differed ($P < 0.0001$), with significant reductions observed in subjects assigned to the HF and IF groups. A significant reduction in insulin concentrations was also observed in the LF group, but this decrease was lower in magnitude than changes in the HF and IF groups (Table 4). Plasma glucose and insulin concentrations at the end of follow-up were significantly ($P < 0.05$) lower in subjects assigned to the HF and IF groups in comparison to those assigned to the LF intervention (Table 4). The estimation of insulin resistance was determined through calculation of an HOMA-IR score. Given the different reductions in fasting glucose and insulin concentration between the 3 treatment groups during the study period, the response of the HOMA-IR score also differed ($P < 0.0001$), with significant reductions observed in subjects assigned to the HF and IF groups but not in those assigned to the LF group (Table 4). The change values of HOMA-IR for HF and IF treatments significantly differed from those for the LF treatment (Table 4). HOMA-IR at the end of study was significantly ($P < 0.05$) better in subjects assigned to the HF and IF interventions in comparison to those assigned to the LF intervention (Table 4). Spearman nonparametric correlation revealed significant relations between changes in plasma insulin and HOMA-IR during the study period and their respective values at baseline ($\rho = -0.363, P = 0.0004$, and $\rho = -0.382$ and $P = 0.0002$, respectively) in the study cohort considered as a whole.

With regard to lipid profile, circulating concentrations of total cholesterol, LDL cholesterol, and triglycerides significantly decreased through the study period and HDL-cholesterol concentrations significantly increased among subjects assigned to the HF and IF interventions, but these did not change among those assigned to the LF group (Table 4). Change values in LDL cholesterol and the HDL- to LDL-cholesterol ratio for HF and IF groups significantly differed from those for the LF group (Table 4). Circulating concentrations of LDL cholesterol at end of the 8-wk intervention were significantly ($P < 0.05$) lower in subjects assigned to the HF group in comparison to those assigned to the LF group (Table 4). HDL- to LDL-cholesterol ratio improved in the HF and IF groups and, to a lesser extent, in the LF group through the study period (Table 4). Changes in circulating concentrations of triglycerides were significantly different in the IF group in comparison to those observed in HF and LF groups (Table 4).

The response of plasma total 8-iso-prostaglandin $F_2\alpha$ to the 3 treatments during the study period differed ($P = 0.0015$), with significant reductions observed among subjects assigned to both the HF and IF groups but not in those assigned to the LF group (Table 4). The change values for the HF and IF groups significantly differed fi
those for the LF group (Table 4). Plasma total 8-iso-prostaglandin F$_{2\alpha}$ concentrations at the end of the study period were significantly ($P < 0.05$) lower in subjects assigned to the HF and IF groups in comparison to those assigned to the LF intervention (Table 4).

$\eta^2$ Values as measures of the magnitude of treatment effects are presented in Supplemental Table 1. All of the above results were confirmed by per protocol analysis (HF, $n = 29$; IF, $n = 29$; LF, $n = 28$), with the exception the loss of statistical significance of the difference in systolic blood pressure at 8 wk between IF and LF groups (data not shown).

**Determinant of cognitive changes after flavanol consumption**

Because of the collinearity between systolic and diastolic blood pressures, only changes in systolic blood pressure were entered in the hierarchical regression analysis because changes in systolic blood pressure were found to be more strictly correlated with changes in composite cognitive $z$ score than were changes in diastolic blood pressure ($\rho = -0.286$, $P = 0.0063$, vs. $\rho = -0.224$, $P = 0.0340$). Changes in HOMA-IR were found to be main determinants of changes in cognitive function, explaining $\sim17\%$ of composite $z$ score variability through the study period ($r^2 = 0.1703$, $\beta = -0.2486$, $P < 0.0001$; Table 6). Changes in plasma isoprostane concentrations and systolic blood pressure explained $\sim4\%$ and $\sim3\%$ of cognitive improvement throughout the study period, respectively (Table 6). Regression analysis with a stepwise approach confirmed this order of relevance of the tested variables on the changes in cognitive function (data not shown).

**TABLE 6**

Hierarchical linear regression in the prediction of determinants of composite cognitive $z$ score changes from baseline in the study cohort$^1$

**DISCUSSION**

In this study, daily CF consumption for 8 wk improved cognitive performance in a group of cognitively intact older adults, without major adverse effects. Furthermore, CF consumption significantly improved blood pressure and several metabolic markers. Interestingly, the improvement in cognitive performance was associated with reduction in insulin resistance, suggesting a possible influential role of glucose metabolism in modulating cognitive function.

The role of cocoa in improving brain health is matter of growing scientific interest (32, 33). Several epidemiologic studies suggest a favorable association between diets rich in flavonoid-containing foods and cognitive function (7–9). Cognitive benefits were also reported in multiple dietary intervention studies (34). Although there has been heterogeneity across studies (34), and some null findings (35), the demonstration of cognitive benefits provides encouraging support for the concept that dietary flavonoids and, specifically, flavanols may play a positive role in maintaining and improving cognitive function, particularly with age.

The current study sheds new light on this relevant topic, providing evidence of a maintained improvement in cognitive performance in response to regular CF consumption; importantly, these benefits were observed at multiple intake amounts. The reported cognitive benefits included improvements in some tasks most affected age-related cognitive decline (1). One important feature of this study was that final cognitive assessments were performed $\sim24$ h after the final drink was consumed, allowing evaluation of the chronic intake effects. This provides evidence that CF-containing foods can be effective in reversing certain aspects of age-related cognit...
decline. These effects were similar to those recently described in elderly individuals with MCI (18). Together these findings suggest a restorative effect of habitual flavanol consumption on different expressions of cognition dysfunction. Consistent with emerging epidemiologic evidence, these data also suggest that regular flavanol consumption may be protective against the development of age-related cognitive dysfunction.

A slight, but significant improvement in performance on the VFT was evident in the LF group. Given the very low CF amount, the observed cognitive improvement was likely a consequence of the intrinsic alkaloids (~50 mg caffeine, 400 mg theobromine). Evidence supports that caffeine, alone (36) and in combination with theobromine (37), can favorably affect cognitive function. It is reasonable then to suggest that the cognitive improvements in the LF group could be attributable to methylxanthine ingestion, highlighting the importance controlling alkaloids in cognitive studies. The more consistent cognitive improvements in the HF and IF group support a direct effect of CFs beyond that simply attributable to the consumption of alkaloids.

Our study provides insights about possible pathways that could underlie the observed cognitive effects. In the and IF groups, there was evidence of improvements in glucose metabolism, with a significant reduction in HOMA-IR, which was a result of decreased circulating insulin and glucose concentrations. Similarly, previous work from our group demonstrated that regular CF ingestion could improve cognitive function and insulin sensitivity in an elderly cohort with MCI (18). These improvements are consistent with a recent meta-analysis: Hooper et al. (19), which indicated promising benefits of CFs on insulin sensitivity. The contributory role of reduction in insulin resistance in the improvement of cognitive performance was 2-fold higher in subjects with MCI than in subjects without MCI (~40% vs. ~17%). These findings are of particular relevance given growing evidence that suggests a central role for insulin resistance in certain aspects of brain aging (38, 39). These findings suggest an influential role of insulin resistance in modulating cognitive function in elderly subjects. Furthermore, these data suggest that dietary interventions that positively affect glucose homeostasis may conf important cognitive benefits. More research in this area is needed to explore this hypothesis further.

CF-mediated improvements in cognitive function have been previously reported (18, 40, 41). A study by Sch et al. (40) showed acute cognitive improvements in healthy adults after the ingestion of drinks with CF amount comparable to what were used in the current study. A single-blind study from Field et al. (41) showed that the consumption of 720 mg CFs (in dark chocolate) resulted in acute improvements in visual and cognitive function in healthy adults. Because improvements in cognitive function have not always been shown (15, 16, 35, 42), a possible consequence of methodologic differences between studies, more research is needed to understand the effect of CF consumption on cognitive function; however, this study and the related CoCoA study performed MCI (18) should offer much-needed insight into the benefits of dietary CFs, particularly among the elderly.

One potential mechanism underlying the observed cognitive benefits may be CF-mediated improvements in cerebral endothelial function. The consumption of 450 mg CFs was shown to acutely improve perfusion in the brain (15). Likewise, the consumption of CFs for 2 wk (at an amount similar to that of the HF group) led to a significant increase in cerebral blood flow in healthy elderly individuals (15). It is thus intriguing to suggest that the observed improvements in cognitive performance could be a consequence of improvements in vascular reactivity. This interpretation of the data fits with previously published studies that demonstrated that acute or regular CF consumption can lead to a significant improvement in nitric oxide–dependent endothelial function within the peripheral vasculature (recently reviewed in reference 19), both in younger and older individuals, as well as in individuals with and without established cardiovascular disease (43). In addition to these effects on vascular function, direct neuroprotective effects of flavanols may be involved. Evidence from in vitro and ani
studies support that flavonoids, including flavanols, can promote neuronal survival and differentiation, effects that may help promote memory and learning (10). Interestingly, in this study, neither blood pressure nor lipid peroxidation changes were shown to contribute significantly to variations in cognitive performance.

The potential clinical relevance and limitations of this study should be considered. First, the moderate benefit cognitive performance and insulin resistance are quite intriguing because they were evident in subjects without evidence of cognitive dysfunction and in a relatively short period of time. Because the intervention lasted just months, the full extent of these cognitive benefits and their duration are not yet known. Furthermore, because only the chronic effects on cognitive function were measured, acute/acute-on-chronic effects of CF consumption could not be determined. Second, subjects in this study were generally in good health, without relevant comorbidities, so this cohort was not representative of all elderly individuals. Third, to avoid confounders, habitual consumers of dietary supplements with antioxidant properties were excluded; however, this is likely have had a limited impact on the generalizability of findings because supplement consumption in our cohort was low (<10%), which is similar to that reported in other cohorts (44). Finally, although measurement of plasma flavanols would have been a better measure of compliance, blood samples were taken after a 12-h fast, well past the expected clearance time of flavanols in plasma (45).

In summary, the results of this study indicate that the regular intake of CFs can improve aspects of cognitive performance among elderly subjects with no evidence of cognitive dysfunction, effects that appear to be dependent on the amount of CF intake. In addition to the possible direct effects of flavanols on cognitive function, general improvements in cardiovascular function and specific metabolic variables could have, alone or in combination, played a role in improving cognitive performance in these subjects. Importantly, these improvements were noted in the context of a typical habitual intake of flavanols (26), as well as in the context of medication use. These findings, together with other published work in a similar cohort (18), provide encouraging evidence that the regular inclusion of flavanol-containing foods may be an effective dietary approach for counteracting cognitive changes associated with brain aging and offer a possible complementary strategy to support cognitive and cardiovascular health with age.

Supplementary Material

Supplemental data:

Click here to view.

Acknowledgments

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Footnotes

5 Abbreviations used: CF, cocoa flavanol; CoCoA, Cocoa, Cognition, and Aging; HF, high flavanol; IF, intermediate flavanol; LF, low flavanol; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; TMT, Trail Making Test; VFT, Verbal Fluency Test.

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